Evaluating the Use of Enclosures to Reintroduce Eastern Indigo Snakes

by

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Abstract

The focus of this thesis is the effects of using enclosures to soft release Eastern Indigo Snakes (*Drymarcon couperi*) for reintroduction. In chapter one, I review the literature for reintroductions, especially papers that have used enclosures to soft release individuals. I also review what is known about the Eastern Indigo Snake's life history, especially topics that might pertain to their successful reintroduction. I also discuss the current status of this species and past reintroduction efforts, including why those efforts failed to establish viable populations. I conclude from this review that no published study has successfully used enclosures to reintroduce snake species, but that this technique has potential to aid in reintroduction of Eastern Indigo Snakes.

In chapter two, I examine spatial ecology and survival to test whether use of enclosures for Eastern Indigo Snakes benefits the establishment of a population. Factors examined include the effects of enclosures on home range size, emigration rate, and home range overlap of males with females. I demonstrate that enclosures have no statistically significant effect on home range size. However, emigration rates are lowered for snakes held in enclosures prior to release. In addition, enclosures increase overlap of male home ranges with those of females. Finally, enclosures have no demonstrable negative affect on survival. These results suggest that using enclosures to soft release Eastern Indigo Snakes alters space use in ways that increase the chances of successfully creating a viable population.

In chapter three, I list major conclusions of this study. In this section the overall effectiveness of using enclosures to reintroduce Eastern Indigo Snakes is reviewed.

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List of Abbreviations

CNF	Conecuh National Forest
CPH	Cox Proportional Hazards Model
MCP	Minimum Convex Polygon

Chapter 1

Introduction

Review of the Reintroductions

Reintroductions are increasingly utilized to conserve imperiled species (Fischer & Lindenmayer, 2000; IUCN/SSC 2013). Reintroductions are projects that involve translocation of individuals of a species to areas within their indigenous range where they have become extirpated (Fischer & Lindenmayer, 2000; IUCN/SSC 2013). The paramount goal of most reintroductions is the formation of a self-sustaining population. However, attainment of this benchmark has varying rates of success. Scientific monitoring of released individuals is thought to improve project success and aid in the development of future reintroduction programs. Survival, reproductive opportunities, and dispersal are considered key factors that should be monitored in reintroductions (IUCN/SSC 2013; Seddon *et al.*, 2007).Two methods employed to release individuals at reintroduction sites are hard release and soft release techniques. A hard release involves freeing the animal directly into the wild. This technique has been used as the sole release mechanism for Eastern Indigo Snake translocations in the past (Smith, 1987; Speake, 1990). However, this technique is associated with the largest cause of reintroduction failure in reptiles where the animals did not acclimate to the release site rapidly enough and emigrated away (Dodd & Seigel, 1991; Germano & Bishop, 2009).

A soft release involves enclosing animals at the release site to acclimate them to their surroundings before allowing them to move freely (Griffith *et al*., 1998). A key variable for this method is the length of time that an individual is constrained and published accounts indicate that this may be as few as 30 days (Pedrono & Sarovy, 2000) to as long as 12 months (Tuberville *et al*., 2005) for tetrapods. While inside the enclosure, animals familiarize themselves with their surroundings so that, once the enclosures are opened, emigrations are minimized and interactions with other individuals near the release site are maximized (Kingsbury & Attum, 2009; Wallace, 2000).

Use of enclosures to soft release individuals in reintroductions has been a popular technique for many vertebrate taxa. Among mammals, enclosures have been used in reintroductions of Black Bears (Eastridge *et al*., 2001), Gray Wolves (Fritts *et al*., 2001), Grey Kangaroos (Campbell & Croft, 2001), primates (Britt *et al*., 2004), Hare Wallabies (Hardman & Moro, 2006), African Wild Dogs (Gusset *et al*., 2006), Western Gorillas (King & Courage, 2008), Water Voles (Moorhouse *et al*., 2009), Riparian Brush Rabbits (Hamilton *et al*., 2010), and Elk (Ryckman *et al*., 2010). Among birds, soft releases have been used for Coots (Anderson *et al*., 2000), Black-eared Miners (Clarke *et al*., 2003), Flightless Rails (Wanless *et al*., 2002), Red-cockaded Woodpeckers (Franzreb, 2004), Whooping Cranes (Nesbitt & Carpenter, 1993), Red-billed Curassows (Bernardo *et al*., 2011), and Prairie Grouse (Snyder *et al*., 1999). Less common in the literature are the use of enclosures to reintroduce reptile species. Nevertheless, this technique has been used on Ploughshares Tortoises (Pedrono & Sarovy, 2000), Hermann's Tortoises (Bertolero *et al*., 2007), Gopher Tortoises (Tuberville *et al*., 2005), European Pond Turtles (Cadi & Miquet, 2004), St. Croix Ground Lizards (Treglia, 2010) and Woma Pythons (Read *et al*., 2011).

Despite the popularity of the soft release technique, few studies have measured its effects. For example, soft releases have decreased active ranges (e.g. Tuberville *et al*., 2005) and excessive emigration (e.g. Ryckman *et al*., 2010) in some species but not others (e.g. Hardman & Moro, 2006). Additionally, soft releases have increased social interactions of released individuals (Gusset *et al*., 2006). Soft release and intensive post release monitoring have been attributed to higher survival in some species (King & Courage, 2008; Bradley *et al*., 2005; Hamilton et al., 2010) but has had no effect on survival for others (Campbell & Croft, 2001). Since different taxa respond differently to release techniques, additional studies of hard and soft releases are desirable for a broad array of taxa, especially reptiles and amphibians (Dodd & Seigel, 1991; Fischer & Lindenmayer, 2000; Germano & Bishop, 2009; Griffith *et al*., 1998).

Very few reintroductions of snake species have occurred. Daltry *et al*. (2001) report a successful use of a hard release to reintroduce the Antiguan racer (*Alsophis antiguae*) to islands formerly occupied by the species. This observation is countered by the unsuccessful use of a hard release to reintroduce *D. couperi* to a variety of sites (Smith, 1987; Speake, 1990; Hart, 2002). Roe *et al*. (2010) examined snake reintroductions pertaining to captive reared vs. wild-caught translocated individuals and reported that captive-reared individuals had decreased survival when compared to their wild counterparts. Only a single published study has examined the efficacy of soft-release techniques to reintroduce snakes and that reintroduction failed because all individuals in the enclosure were depredated (Read *et al*., 2011).

Background for Study Species

In this study, I compare hard and soft release techniques as tools for the reintroduction of Eastern Indigo Snakes (*Drymarchon couperi*) to the Conecuh National Forest of Alabama. Here, I review pertinent information on the life history of the study organism.

Eastern Indigo Snakes are one of the largest native snake species in the United States with a record length of 262.9 cm (Conant & Collins, 1998). The Eastern Indigo Snake's geographic range is limited to the lower latitudes of the southeastern US. Historically known from parts of South Carolina, Georgia, Florida, Alabama, and Mississippi, wild populations of the species are now restricted to portions of Georgia and Florida. In the northern parts of its range it is most often associated with longleaf pine (*Pinus palustris*) ecosystems (Speake, 1993). In these areas Eastern Indigo Snakes are known to be a commensal of another longleaf specialist species the Gopher Tortoise (*Gopherus polyphemus*) (Holbrook, 1842; Lawler, 1977; Landers & Speake, 1980; Speake & McGlincy, 1981; Stevenson *et al*., 2003; Hyslop *et al*., 2009a). The longleaf pine and its associated flora and fauna make up one of the most imperiled ecosystems in the United States. With less than 3% of the original longleaf ecosystem left (Noss & Peters, 1995; Ricketts *et al*., 1999; Van Lear *et al*., 2005), serious losses in habitat have caused declines of Gopher Tortoises, a keystone species of longleaf pine forests, throughout much of their range. This coupled with over collection for the pet trade and gassing of tortoise burrows for rattlesnake roundups caused the Eastern Indigo Snake to be listed in 1978 as a Threatened Species by the US Fish and Wildlife Service.

Eastern Indigo Snakes have the largest documented home range of any native North American snake species, with home range size being as large as 1,500 ha (Hyslop, 2007).

Translocated juvenile snakes have been known to use an area of up to 100 ha (Smith, 1987). Males occupy larger home ranges than females (Breininger *et al*., 2011; Hyslop, 2007; Moler, 1985; Smith, 1987). Hyslop (2007), in a study of free-ranging Eastern Indigo Snakes near the source population of snakes used in this thesis, reported average MCPs for males of 538 ha, and for females of 354 ha. Breininger *et al*. (2011), in a study from central Florida, reported average MCPs of 118 ha for males and 41 ha for females. Moler (1985), in northern Florida, reported average MCPs of 141 ha for males and 22 ha for females. Large home ranges are thought to affect survival rates negatively by increasing the chances that a snake will encounter roads, predators, or diseases and parasites (Hyslop, 2007). That study also reported annual survival rates for wild Eastern Indigo Snakes to be between 0.72-0.89. A recent study of indigo snake survival near and on the source sites of the snakes used in this study showed survival estimates of 0.70 for adults to 0.52 for subadults, with survival being positively correlated with body size (Hyslop *et al*., 2011). In a study of translocated juvenile indigo snakes, Smith (1987) found that survival was between 0.61 and 0.89 for a two-month time period, with yearlings having a significantly higher survival rate than hatchlings.

Large home ranges also appear to be associated with the fact that Eastern Indigo Snakes require a variety of habitat types within their annual cycle of activities. Habitats used by these snakes include upland longleaf-dominated sandhills and associated low wetlands such as ephemeral ponds, bayheads, and creek drainages (Hyslop *et al*., 2009a; Smith, 1987; Speake *et al*., 1978; Stevenson *et al*., 2003). It has been suggested that factors such as seasonal shifts in refugia and foraging cause Eastern Indigo Snakes to use large home ranges that encompass many varied habitat types (Hyslop *et al*., 2009a). It is also suggested that males require larger home ranges than females as part of their efforts to locate mates (Brown & Weatherhead, 1999). Thus, while incurring a cost of increased mortality associated with large home range size, males also may gain a benefit in increased mating opportunities by increasing the area of their home range, especially if they can detect the presence of neighboring females.

Eastern Indigo Snakes are known to use many types of refugia, presumably to protect themselves from predators and thermal extremes. In winter months, Eastern Indigo Snakes are most often associated with Gopher Tortoise burrows. In one study, 90% of winter refugia were tortoise burrows (Hyslop, 2007). However, other refugia, such as windrows, stump holes, and human refuse, have emerged as important summer shelters (Hyslop *et al*., 2009a; Speake *et al.*, 1978). Eastern Indigo Snakes are generalist predators that are known to eat mammals, birds, amphibians, and reptiles. They are particularly ophiophagous, consuming venomous species (Stevenson *et al*., 2003; Stevenson *et al*., 2010). Declines in Gopher Tortoise populations, increased mortality due to the gassing of their burrows for rattlesnake round ups, destruction of the many varied habitats which encompass the snake's refugia and prey needs, and over collection for the pet trade have led to the extirpation of Eastern Indigo Snakes from Alabama (Speake *et al*., 1982). However, recent legislation prohibiting the gassing of burrows, active longleaf ecosystem restoration efforts, and protected status banning collection of these snakes offers the potential for their repatriation.

Currently the Eastern Indigo Snake in Alabama is protected by the state of Alabama and is listed as a species of highest conservation need (Godwin, 2004). Historically, the species inhabited the Lower Coastal Plain of Alabama (Mount, 1975), with the last documented wild specimens having been observed by Neill in 1954 (Hart, 2002). From the mid-1970s through the 1980s, the U.S. Fish and Wildlife Service, Alabama Cooperative Fish and Wildlife Research Unit coordinated efforts to rebuild declining populations through supplementation of dwindling populations (Speake, 1990). In 2002, a review of the status of Eastern Indigo Snake proposed them to be incredibly rare and possibly extirpated in Alabama. At that time, the last reported, but unconfirmed, record of the species from Alabama occurred in 2000 (Hart, 2002). Recent surveys of the supplemented sites have failed to document the presence of these snakes (Clay, 2006, 2007; Guyer *et al*., 2007; Rall, 2004), suggesting that supplementation failed to maintain any viable population. In 2008 efforts to try another reintroduction in Alabama were coordinated between Auburn University and several partner agencies and organizations (Godwin et al., 2011). This thesis is based upon that study.

Chapter 2

Evaluating the Use of Enclosures to Reintroduce Eastern Indigo Snakes

Abstract

Reintroductions are a common tool used for the conservation of imperiled species worldwide. Use of enclosures to effect soft release of individuals for reintroductions has become commonplace because this technique limits dispersal and increases chances for socializations leading to reproduction. However, no published study has used this technique for snake reintroductions. In this study we examined the efficacy of using enclosures to soft release Eastern Indigo Snakes (*Drymarchon couperi*) within the Conecuh National Forest, a release site at the northern limit of the geographic range for this species. During 2010 and 2011, 38 Eastern Indigo Snakes were reintroduced using both hard (n = 20) and soft (n = 18) release techniques. The snakes were subsequently tracked using radio telemetry. Data gathered from radio telemetry were then used to evaluate the release techniques. We performed analyses on home range size and emigration rates for hard- and soft-released individuals paired to control for effects of sex and time. We also compared male-female overlap between the two release groups. Further, we examined whether the enclosures affected snake survival. Release type did not affect home range size, but did decrease the rate at which snakes emigrated from the release site. Soft-released male snakes had a higher percentage of overlap with females than hard-released males. Finally,

enclosures did not significantly affect survival. Our study indicates that using enclosures on large snake species alters movements in ways that should increase viability of reintroduced populations.

Introduction

Reintroductions are increasingly commonplace in efforts to conserve imperiled species (Fischer & Lindenmayer, 2000). Two prevailing methods used to release individuals at reintroduction sites are hard release and soft release techniques. A hard release involves freeing the animal directly into the wild. A soft release involves enclosing animals at the release site to acclimate them to their surroundings before freeing them. Soft releases are recommended because they minimize excessive dispersal, allow acclimatization to the local environment, increase foraging efficiency, and create reproductive opportunities (Kingsbury & Attum, 2009; Wallace, 2000). Soft-released animals become familiar with their surroundings so that, once released, dispersal distances are minimized and opportunities for interactions with other animals close to the release site are maximized. In practice, these expectations are not always realized. For example, soft release decreases active ranges after release in some species (e.g. Tuberville *et al.*, 2005) but not others (e.g. Hardman & Moro, 2006). While studies on soft release techniques have been employed for many mammal, bird, and turtle species, no successful study on the effectiveness of using enclosures to soft release snake species has been published (Read, Johnston & Morley, 2011). Additional studies of hard and soft releases are desirable for a broader array of taxa (Seddon, Armstrong & Maloney, 2007; Fischer & Lindenmayer, 2000), particularly for reptiles (Dodd & Seigel 1991; Germano & Bishop, 2009) which are becoming increasingly imperiled (Gibbons *et al.*, 2000).

Loss of habitat has led to declines of numerous reptile species (Gibbons *et al*., 2000), especially species, such as the Eastern Indigo Snake (*Drymarchon couperi*), that are tied to specific habitats (Guyer & Bailey, 1993). The geographic range of the Eastern Indigo Snake is limited to the Lower Coastal Plain of the southeastern United States. In the northern parts of its range it is most often associated with longleaf pine (*Pinus palustris*) ecosystems (Guyer & Bailey, 1993). The longleaf pine and its associated flora and fauna constitute one of the most imperiled ecosystems in the United States. With less than 3% of the longleaf ecosystem left (Noss & Peters, 1995; Ricketts *et al.*, 1999; Van Lear *et al*., 2005), serious losses in habitat have caused marked declines of Easter Indigo Snakes throughout much of the geographic range of the species. Due to these declines, the species was listed as Threatened (U.S. Fish and Wildlife Service 1978). Within the longleaf ecosystem, Eastern Indigo Snakes use many varied habitats that include upland, longleaf-dominated, sandhills and associated low wetlands such as ephemeral ponds, bayheads, and creek drainages (Hyslop, Cooper, & Myers, 2009a; Smith, 1987; Speake, McGlincy, & Colvin, 1978; Stevenson, Dyer, & Willis-Stevenson, 2003). One reason that these snakes utilize so many varied habitats is their broad diet. Eastern Indigo Snakes are generalist predators that are known to eat mammals, birds, amphibians, and reptiles. They are particularly ophiophagous, often consuming venomous species (Stevenson *et al*., 2003; Stevenson *et al*., 2010). It has been suggested that factors such as seasonal shifts in habitat use and prey availability cause these snakes to use large home ranges (Hyslop *et al*., 2009a).

Eastern Indigo Snakes have the largest documented home range of any snake species native to North America, with reported home range sizes in adults as large as 1,500 ha (Hyslop, 2007). Translocated juvenile indigo snakes have been known to use an area of up to 100 ha (Smith, 1987). Males occupy larger home ranges than females (Hyslop, 2007; Smith, 1987), which may enhance their efforts to gain mates (Brown & Weatherhead, 1999). However, large home ranges can negatively affect survival, especially in increasingly fragmented landscapes (Breininger *et al*., 2012). Thus, while incurring a cost of increased mortality, males may gain a benefit in increased mating opportunities by increasing the area of their home range, especially if they can detect the presence of neighboring females.

While male and female Eastern Indigo Snakes differ in home range size, sex does not appear to affect survival rates in wild populations (Hyslop *et al*., 2009b). However, other factors do affect survival in Eastern Indigo Snakes. Hyslop *et al.* (2011) found that annual survival probability for wild snakes ranges from 0.70 for adults to 0.52 for subadults, with survival being positively correlated with body size. In a study of translocated juvenile indigo snakes, Smith (1987) found that survival was between 0.61 and 0.89 for a two-month time period, with yearlings having a significantly higher survival rate than hatchlings.

In snake translocations, appropriate use of refugia has been linked to a snake's ability to survive (Roe *et al.*, 2010) and could play an important role in successful reintroductions of Eastern Indigo Snakes. These snakes are known to use many types of refugia, presumably to protect themselves from predators and thermal extremes (Hyslop, 2007). In the longleaf ecosystems, Eastern Indigo Snakes are well known to utilize Gopher Tortoise (*Gopherus polyphemus*) burrows as vital refugia (Holbrook, 1842; Lawler, 1977; Landers & Speake, 1980; Speake *et al*., 1978; Speake & McGlincy, 1981; Stevenson *et al*., 2003; Hyslop *et al*., 2009a), especially during winter months (Hyslop, 2007). During summer, windrows, stump holes, and human refuse, are important shelters (Hyslop *et al*., 2009a; Speake *et al*., 1978).

Declines in Gopher Tortoise populations, increased mortality of snake commensals due to the gassing of tortoise burrows for rattlesnake round ups, destruction of the varied habitats upon which snakes depend, and over collection for the pet trade have led to the extirpation of Eastern Indigo Snakes from Alabama (Speake & McGlincy, 1981). However, recent legislation prohibiting the gassing of burrows, active longleaf ecosystem restoration efforts, and the presence of recovering populations of Gopher Tortoises makes Alabama an appropriate state for reintroduction. In Alabama, the Eastern Indigo Snake is protected and listed as a species of highest conservation concern (Godwin, 2004). From the mid 1970's through the 1980's, the U.S. Fish and Wildlife Service and the Alabama Cooperative Fish and Wildlife Research Unit coordinated efforts to rebuild declining populations through supplementation of dwindling populations. Recent surveys of the supplemented sites failed to document the presence of Eastern Indigo Snakes, suggesting that conservation efforts failed to maintain viable populations.

This study is part of a second effort to reintroduce Eastern Indigo Snakes to Alabama. In it we perform an experiment to determine whether the use of enclosures (soft release) improves patterns of home range size, home range overlap, and survival when compared to patterns associated with hard-released snakes. For soft release to be a viable conservation strategy for Eastern Indigo Snakes, we expected that individuals released from enclosures would have smaller home range areas and lower dispersal rates compared with hard-released individuals. Additionally, we expected that soft-released males would have a larger cumulative percentage of their home range that overlaps with females. However, such benefits of soft releases might be lost if survival decreased for penned individuals. Therefore, we also tested for differences in survival rates of soft- and hard-released snakes.

Methods

Our study was conducted in the Conecuh National Forest (CNF) in southern Alabama, United States. The last wild Eastern Indigo Snake documented in Alabama, by Neill in 1954, came from an area within approximately 15 km of the CNF (Mount, 1975), suggesting that this species occurred on our study site within the historical past. For the past 30 years CNF has maintained and restored the longleaf pine ecosystem through use of prescribed fire, plantings, and stand thinning. For this reason and because the US Forest Service was a willing partner, we selected the CNF as our study site.

Individuals used for reintroduction were reared in captivity from eggs laid by wild-caught gravid females. Gravid female snakes were collected from locations in southern Georgia (Figure 1) and were transported to Auburn University, where they were held until they laid their eggs (58-105 days). Each female was then returned to its point of capture and released. Eggs were incubated (101-122 days) in temperature-controlled plastic containers containing damp cloth to maintain moisture. Once hatched, neonate snakes were reared in captivity for approximately 1.75 years. Prey were provided weekly and varied (snakes, lizards, frogs, fish, mammals, or birds), depending on what each individual would accept. Approximately 2 months prior to release, radio transmitters were surgically implanted into the coelomic cavity of each snake and these snakes were then placed singly into 4-meter diameter outdoor enclosures where they were fed and monitored for a month after surgery. Because no medical problems were observed in these snakes, they were then released to the CNF.

The release site is located in the CNF within the Blue Springs Wildlife Management Area and consists of xeric upland sandhills that grade into aquatic flood plains of a headwater stream (Pond Creek). The sandhill is comprised of fire-maintained longleaf pine and mixed pine hardwood forests. It also contains several old wildlife food plots dominated by warm season grasses and occupied by several Gopher Tortoises.

The soft release site consisted of six adjoining enclosures that were 0.52-1.01 ha in size (mean of 0.78 ha. Each enclosure was made of 1.25 meter tall, 6 mm mesh, hardware cloth that was buried approximately 15 cm into the ground. Inside each enclosure, four 2.5 m diameter plastic pools were embedded so that they were flush with the soil surface; these were filled with water. Woody stems < 15 cm in diameter were cut at ground level and used to create brush pile refugia in each pen. At least three Gopher Tortoise burrows were present within each enclosure and at least one Gopher Tortoise resided in each pen. Prior to release, prey items, such as snakes and frogs, were stocked in each enclosure. After release, penned Eastern Indigo Snakes received additional prey in the form of an individual snake or small mammal approximately once a week. This was done by tracking the snake to its location within a pen and, if above ground, presenting a prey item directly to the snake. If the snake was underground, then the prey was released into the snake's underground refugium. Construction and maintenance of the enclosures constituted hundreds of man/hours and considerable cost.

In 2010, 17 snakes were released; eight were released into the enclosures and nine were hard-released. The eight soft-released snakes were distributed so that two enclosures held male-female pairs and four enclosures contained a single snake each. Paired snakes were matched for size but were never from the same clutch. In 2011, 21 snakes were released, with 11 hard released and 10 soft released. The soft-released snakes included a male-female pair (as above) in two enclosures, a male-male pair in one enclosure, a female-female pair in one enclosure, and two enclosures with a single snake (one male and one female). As much as possible, snakes within each clutch were distributed evenly between the soft- and hard-released categories. Hard-released snakes were placed around the perimeter of the enclosures.

After release, each snake was tracked using radio telemetry approximately three times a week from May – August, and once a week for the rest of the year. Snakes were recaptured in February and March of 2011 and 2012 to replace transmitters. Snakes were tracked to their exact location and an effort was made to observe the snake if it was above ground. A Global Position System with 3 m accuracy, was used to map telemetry locations. Locations were plotted in ARC GIS, which was used to construct Minimum Convex Polygons (MCPs); these delineated home range areas for further analyses. The MCP method was used due to its standardized method of calculation between all individuals eliminating user bias in constructing home range areas. The MCP method has also been shown to perform similarly if not better than other methods of home range calculation for herpetofauna (Row *et al.*, 2006; Miller, 2008). For snakes that died underground, efforts were made to confirm this by either digging or using a burrow camera to determine the fate of the snake. If the snake died under ground, then the last time that it was seen was used as the last known time it was alive, with death attributed to the next consecutive tracking day. All snakes placed in enclosures eventually escaped via rodent burrows and other underground routes. Nevertheless, soft-released individuals stayed in the pens a mean of 62 days (5-190 days). For paired design tests described below, snakes that spent less than thirty days in enclosures were considered to have had insufficient time to gain the experience of a soft release and were excluded from further consideration. All statistical tests described below, unless otherwise cited, were performed in SAS 9.2 (SAS Institute, Cary, North Carolina) with alpha set at $p = .05$.

Home Range Analysis

Since hard- and soft-released snakes were freed or placed in pens on the same day and soft-released snakes exited the enclosures at differing times, hard released snakes potentially had a “head start” on forming home ranges. Additionally, several hard-released snakes became undetectable either because the transmitter battery failed or because the individual dispersed too far to be detected. To account for this, data for each hard-released animal were truncated to fit a soft-released animal monitored for the same period of time. Since males have larger home ranges than females, males and females were paired separately from each other. This procedure created a design in which individuals were paired to maximize their overlapping time span. An MCP was created for each snake using the points for each pair’s overlapping time span. MCPs are sensitive to number of relocations, so with tracking rate relatively equal and overlapping the time span we generated pairs of hard- and soft-released snakes that had similar numbers of observations for both individuals. We then truncated pairs excluding those where the soft release individual experienced less than 30 days in the enclosures. This gave us three pairs of males and six pairs of females for further analyses. Because assumptions of normality were met, the difference of the areas used within each pair was examined with a paired T-test. Since home range varies by sex, pairs were further examined by testing each sex separately. Again because of low sample size and data that did not greatly vary from normal pair T-tests were used to compare differences. A consistent decrease in home range size for penned snakes was considered to be consistent with the hypothesis that penning improves establishment of home ranges in Eastern Indigo Snakes. Any other relationship was considered to be inconsistent with this hypothesis.

Dispersal

The rate at which snakes emigrated from the release site was used to examine the enclosure's effects on dispersal. This rate was calculated for each individual by regressing the cumulative maximum distance moved from the point of release against time since release for all relocations of that individual. The slope of this regression was used as an estimate of the overall rate of emigration for that individual. Hard- and soft-released individuals were paired based on, sex and overlapping numbers of relocations, since rate was correlated to the number of relocations. Data was then equalized by sex with three pairs of males and three pairs of females in this analysis. These paired data failed the assumptions of normality, so a Wilcoxon Sum Rank Test was used to test for differences in emigration rates between the release types. A significant difference, with soft-released snakes having slower emigration rates, was considered to be consistent with the hypothesis that penning improves relocation of Eastern Indigo Snakes. Any other relationship was considered to be inconsistent with this hypothesis.

Male and Female Overlap

We used data gathered in 2010 (n = 8 females; n = 9 males) to examine the hypothesis that use of enclosures increased the overlap between male and female snakes compared with hard-released animals. Only data from 2010 were used for this analysis since this was the only year during which all snakes released were monitored using telemetry.

We calculated overlap as the percentage of each male snake's MCP that overlapped each female snake and summed these values to get a cumulative percent overlap for all females. Because the data were normally distributed but had unequal variances, a Satterthwaite pooled T-

Test was used to determine if there was a significant difference between hard- and soft-release categories. As an alternative test, a regression, with percentage overlap (dependent variable) and time spent in an enclosure (independent variable) was used to test whether percent overlap was correlated with the amount of time males spent in an enclosure. A significant soft release t value and a positive regression correlation were considered to be consistent with the hypothesis that penning improves reintroduction of Eastern Indigo Snakes. Any other relationship was considered to be inconsistent with this hypothesis.

Survival

Survival was estimated over 10 consecutive months for 2010 (June to March) and 2011 (May to February). This variable was estimated using a Kaplan-Meier procedure (Hutchon, 2006). Data started when a snake was released and ended when it was recaptured for transmitter replacement or known to have died. Snakes that experienced transmitter failure or that emigrated so far from the area that they could not be relocated were censored (last observation date before contact was lost). A Cox Proportional Hazards (CPH) model was used to test the effect of sex, home range size, maximum dispersal distance, release type, and amount of time spent in an enclosure (independent variables) on survival (dependent variable). The first model was run using only known mortalities as events. However, a second model was created in which snakes that emigrated long distances from the release site and never returned were treated as mortalities since they were no longer a functional part of the population. For both tests, a significant negative correlation was considered to indicate a detrimental effect of penning on snake reintroduction. Any other relationship suggested no known detrimental effect of penning.

Results

MCPs created using the total locations for all snakes varied between 0.9 and 493.9 ha, with an average of 108.3 ha. Female MCPs ranged from 0.9 to 276.5 ha, with a mean of 60.9 ha. Male MCPs ranged from 1.31-493.9 ha and averaged 159.4 ha.

Although soft-released snakes (mean =30.76, 95%CI $\pm$ 13.01) tended to have larger home range sizes than hard-released snakes (mean =22.43, 95%CI $\pm$ 11.02) (Figure 2), this trend was not statistically significant ($t = 1.01$, $p = 0.17$) and was opposite the expected direction. When we segregated the sexes, soft release females (mean = 20.57, 95%CI $\pm$ 11.68) did not differ significantly ($t = -0.88$, $p = 0.21$) from hard release females (mean = 25.89, 95%CI $\pm$ 16.04). However, soft release males (mean = 51.13, 95%CI $\pm$ 12.42) did have significantly larger MCPs ($t = 4$, $p = 0.028$) than hard release males (mean = 15.52, 95%CI $\pm$ 5.68).

Emigration rates of soft-released snakes (mean = 2.68, 95%CI $\pm$ 1.04) were significantly ($z = -2.16$, $p = 0.01$; Figure 2) lower than those of hard-released individuals (mean = 29.80, 95%CI $\pm$ 26.86).

The cumulative percentage of a male's home range that overlapped with female home ranges was significantly greater ($t = -2.88$, $p = 0.05$; Figure 3) for soft-released males (mean = 288.6, 95%CI $\pm$ 489.66) than hard-released individuals (mean = 61, 95%CI $\pm$ 48.10). Similarly, there was a significant positive relationship between the time that a male snake spent in an enclosure and the cumulative percentage of home range overlap with female snakes ($R^2 = 0.76$, $p= <0.01$; Figure 4).

For the 17 individuals released in 2010, six were known to have died, five were censored, and six were known to have lived to the end of the study. These data produced a survival probability of 0.54 ± 0.44 (95% CI) over the snakes' first active season (10 months). For the 21 individuals released in 2011, nine snakes were known to have died, six were censored, and 6 were known to be alive at the end of the study. This produced a survival probability of 0.49 ± 0.37 (95%CI).

Results of the CPH model showed no significant effects of any variable on survival when mortality was restricted only to animals known to have died. Although sex, with females having higher survival (hazards ratio 0.38) and release type with soft release snakes having lower chances of surviving (hazards ratio 2.80) approached significance (Table 1). Results of the CPH model showed several significant variables with regards to survival when emigrants were included as known mortalities. In this model sex had the strongest significant effect on survival, (chi-square = 8.70, $p = <0.01$) with females being more likely than males to survive (hazards ratio = 0.23). Home range size also had a significant effect on survival (chi-square = 4.79, $p = 0.03$) because snakes with larger home ranges experienced lower survival. However, a hazards ratio of 1 indicated that it would take a large increase in home range size to affect survival. Similarly, the effect of dispersal distance on survival approached significance, with longer dispersal distances being associated with slightly lower survival again though with a hazards ratio of 1 it would take a large increase distance to affect survival. Release type showed that again soft release had increased mortality (hazards ratio = 2.00) however, this trend was not significantly supported. Time spent in an enclosure had no significant influence on survival (Table 2).

Discussion

Our data document that use of soft-release for reintroduction of Eastern Indigo Snakes to conservation lands yields movement and survival patterns that increase the likelihood of project success. However, this is a benefit largely associated with male snakes. Females, regardless of release type, tend to remain on relatively small home ranges near the release site, a pattern that could cause rapid accumulation of this sex. Males, whether soft released or not, tend to have larger home ranges than females. Compared with hard-released males, soft-released males tended to have larger home ranges and these home ranges tended to remain closer to the release site. These features of male movements increased mating opportunities for soft released males because they greatly increased home range overlap with females. Thus, based on spatial ecology alone, benefits of soft release are created by behavioral changes in primarily males.

I found no significant effect of penning on survival of the snakes. While the soft release snakes seemed to have lower survival this trend is not significantly supported and further corroborated since no correlation was found between length of time in enclosures and survival. Survival of females was significantly greater than that of males. This appears to result from the fact that males moved longer distances, a feature that should expose them to more sources of mortality (Breininger *et al*. 2012). Survival estimates for this reintroduction were similar to or higher than those other studies of snake reintroductions (Read *et al*., 2011; Smith, 1987) More importantly, survivorship of the snakes at the release site was similar to those of free-ranging Eastern Indigo Snakes from the source of the snakes used in this study (Hylsop *et al*., 2011). This provides additional evidence to suggest that using enclosures has no detrimental effect on the penned animals. No mortalities were associated with cannibalism in the enclosures despite six instances in which we penned two snakes together. Therefore releasing multiple individuals into one enclosure did not seem to adversely affect the reintroduction and suggests that future use of soft releases in snake conservation might be performed with a single.

As reintroductions of animals become more commonplace, science-based studies examining the effects of release techniques on the overall success of projects are needed. Eastern Indigo Snake reintroductions are being planned elsewhere across the range of the species. As these projects to move forward, an understanding of how to properly release individuals will be vital to project success. In this study I showed that, at least for the first two years of the project, use of soft release techniques improved space use in ways that enhanced the chances of establishing a viable population. Soft release techniques seem to decrease excessive dispersal, the rate of emigration, and increase reproductive opportunities, with no significant cost to the survival of the released individuals. The findings of this study indicate that using enclosures to release snakes should be a recommended strategy when reintroducing indigo snakes.

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Variables	Chi-square Value	P Value	Hazards Ratio
Sex	3.08	0.07	0.38
Release Type	2.98	0.08	2.8
Days in Enclosures	1.50	0.22	1
Home Range	1.16	0.28	0.99
Distance Moved	1.24	0.26	1

Table 1. Cox Proportional Hazard model including only known mortalities.

Variables	Chi-square Value	P Value	Hazards Ratio
Sex	8.7	<0.01	0.23
Release Type	2.30	0.12	2.04
Days in Enclosures	0.21	0.63	1
Home Range	4.79	0.02	1
Distance Moved	3.31	0.06	1

Table 2. Cox Proportional Hazard model including emigrants as mortalities

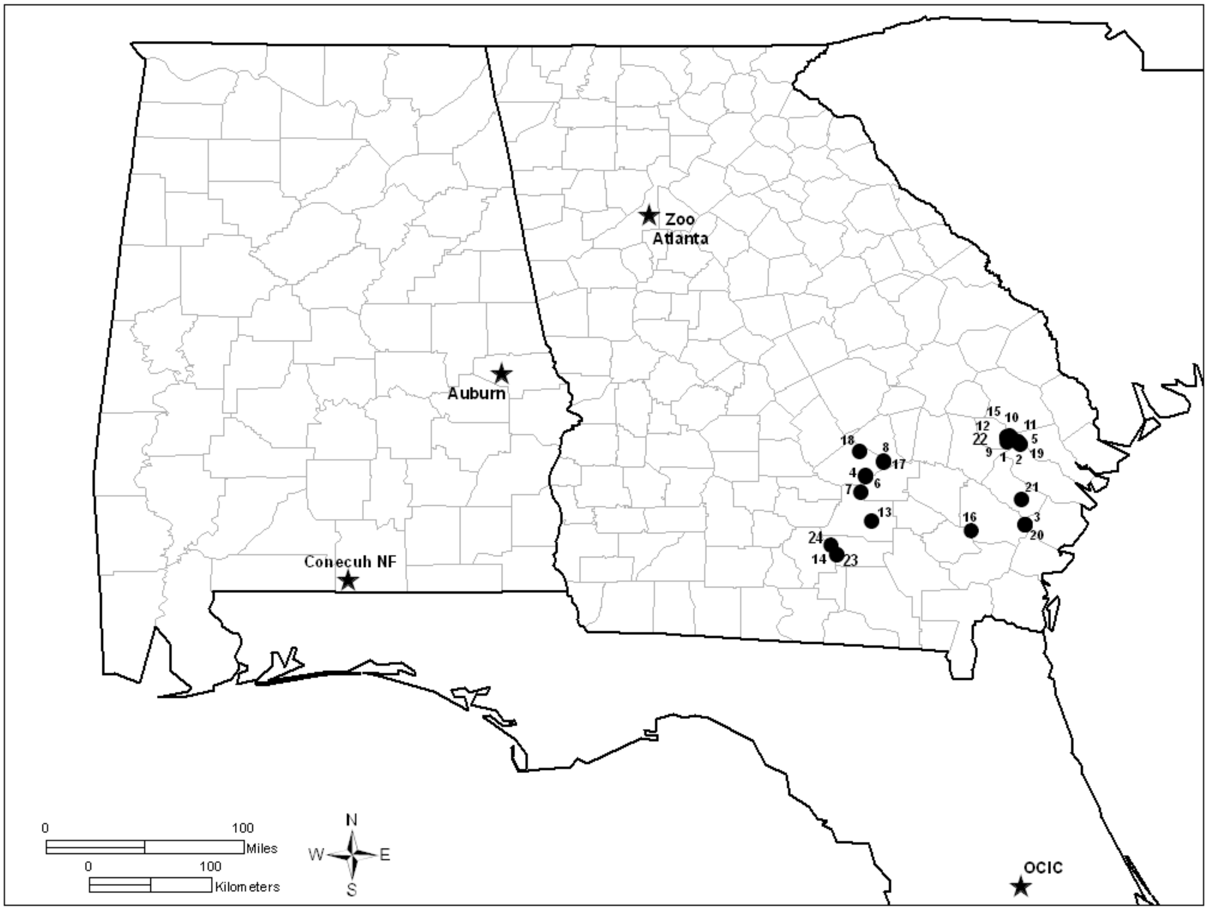

Figure 1. Map of important study locations. Solid circles indicate sources of gravid females, Stars indicate locations of captive rearing (Zoo Atlanta and Auburn), and release of relocated snakes (Conecuh NF).

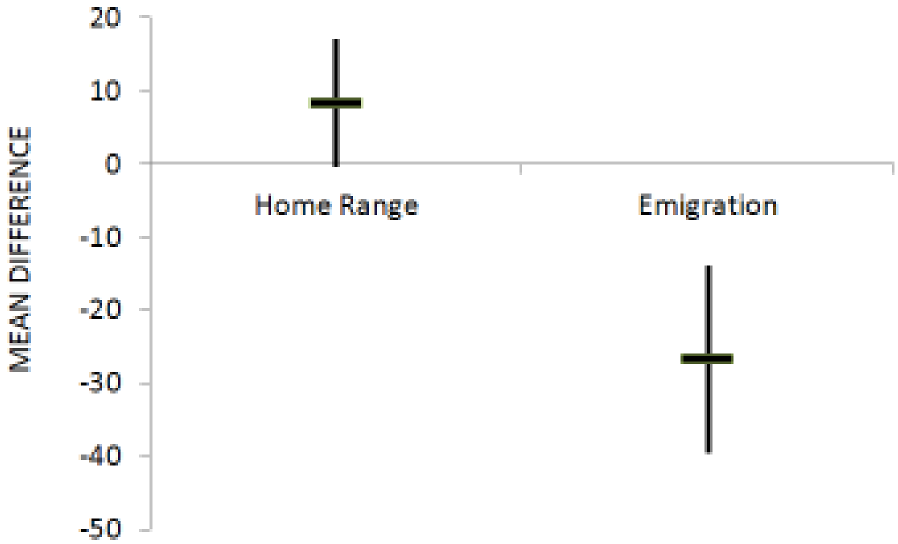

Figure 2. Mean difference in pairs for home range and emigration rates between hard- and soft-released Eastern Indigo Snakes. Pairs are matched by sex and for length of time after release from pen.

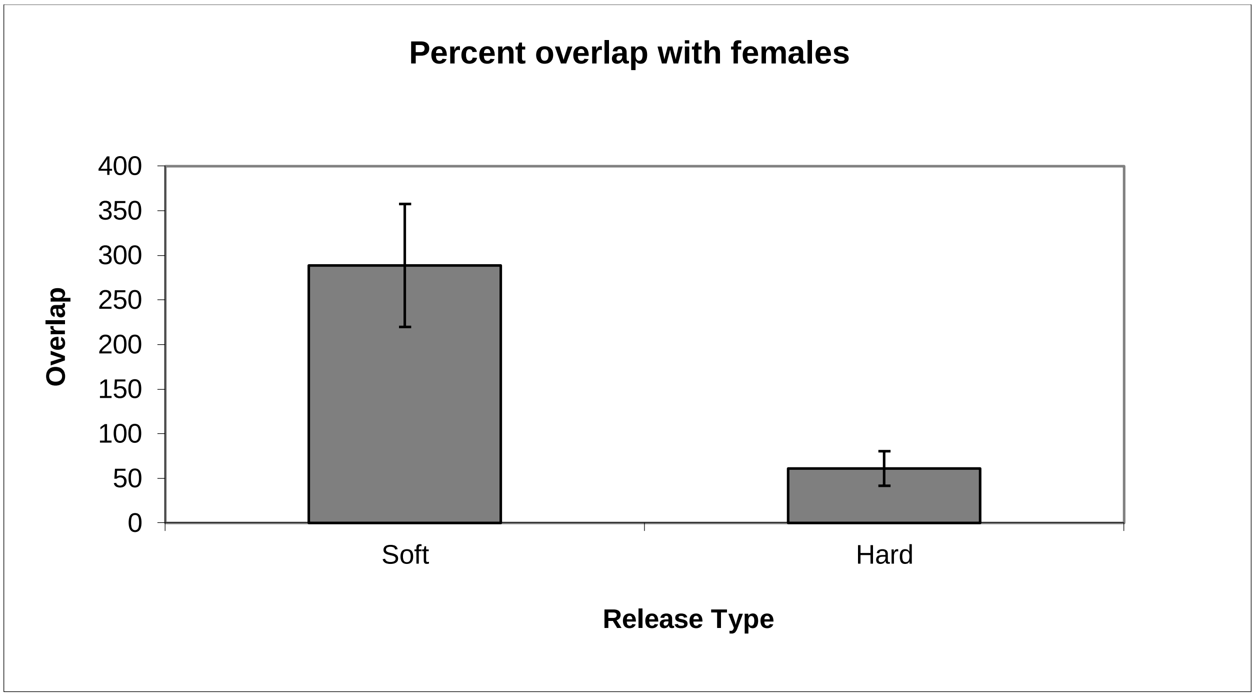

Figure 3. Cumulative percentage of male's home range that overlaps females.

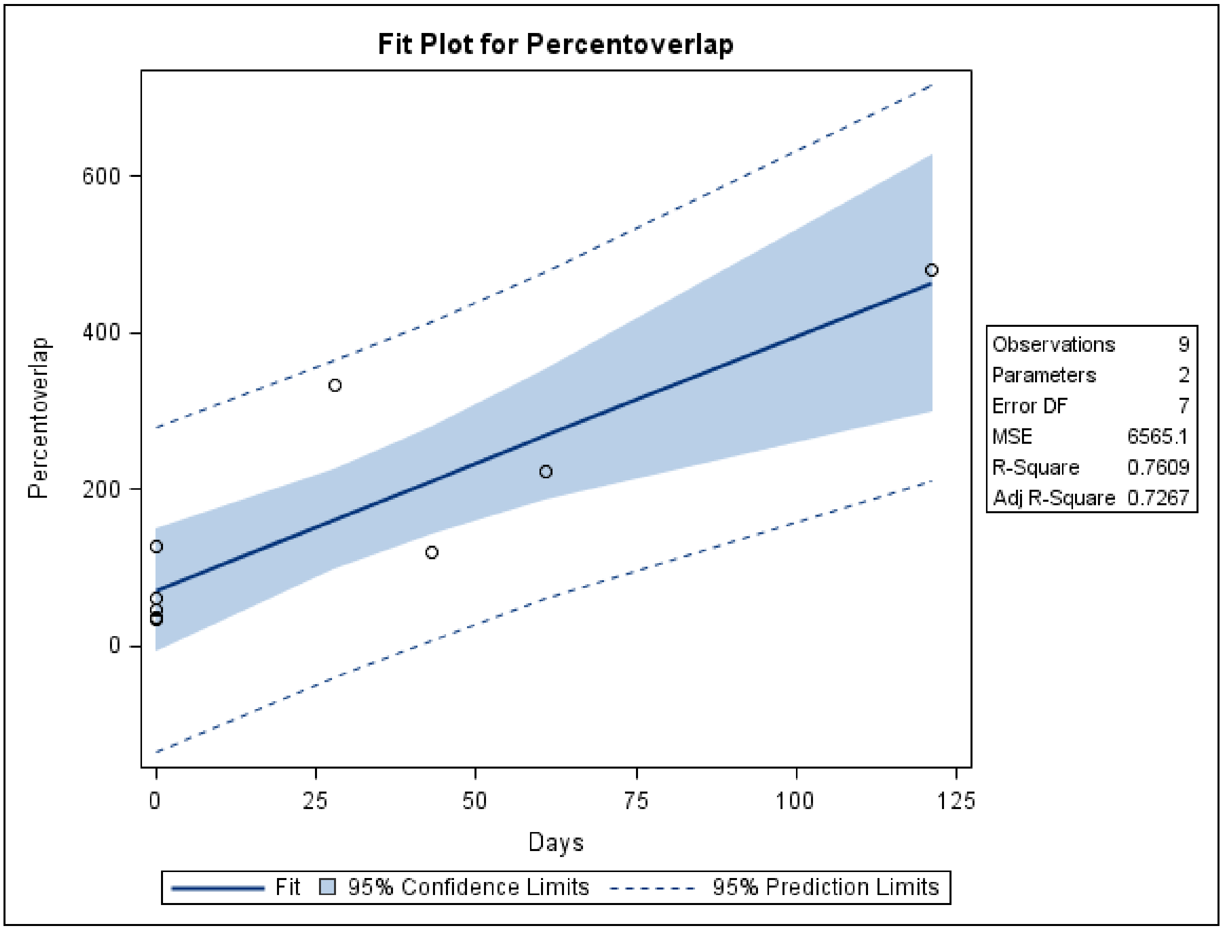

Figure 4. Regression of cumulative percent overlap of a male's home range with females on time spent in an enclosure. Solid line is least squares linear fit, blue shading is 95% confidence limits, and dotted lines are 95% prediction limits.

Chapter 3

Conclusions

This study demonstrated that using enclosures to soft release Eastern Indigo Snakes for reintroduction alters space use by male snakes in ways that will likely improve the success of a reintroduction. However, the paired comparison showed no significant difference between the hard and soft release techniques overall, yet when segregated by sexes the soft released males might actually increase their active range size. However, using enclosures seems to slow the rate at which snakes travel away from their release site.

Also hard released males overlapped a smaller percentage of female home ranges than soft released males. These findings indicate that using enclosures to release indigo snakes potentially increases reproductive possibilities for the founding population.

No mortalities were associated with cannibalism in the enclosures. Therefore releasing multiple individuals into one enclosure did not seem to adversely affect the reintroduction and might be a preferred method of soft release.

Overall, soft releasing snakes did not seem to affect snake survival. While soft release snakes did seem to have lower survival this trends likelihood was not significantly supported. Support for the idea that the enclosures decreased survival is further uncorroborated in the fact that the amount of time a snake spent in the enclosure had no effect on survival. However, males, likely because of their larger home ranges, had significantly lower survival.

Survival in this study was comparable to survival of free-ranging snakes at the source populations and was similar to or higher than other reintroductions of snake species.

The enclosures used in this project were quite large, which made them costly and difficult to construct and maintain. The difficulty in maintaining them likely increased the snakes' ability to prematurely escape. Future reintroductions that use enclosures might consider building smaller enclosures and burying the fences at least 1.5 meters in the soil to minimize premature soft release.

Beneficial effects on snake movement patterns and a lack of significant negative effects on snake survival indicate that enclosures should be a recommended method for the reintroduction of large snakes like the Eastern Indigo Snake.

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